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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ETRAVIRINE IN ITS BULK DOSAGE FORM BY USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD AS PER INTERNATIONAL CONFERENCE ON HARMONISATION GUIDELINES

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ABSTRACT

Objective: An accurate, precise, rapid and economical reverse phase high performance liquid chromatography (HPLC) method has been developed and validated for the estimation of etravirine in pharmaceutical dosage forms, using an ultraviolet detector.

Method: Elution was carried out using a mobile phase consisting of HPLC grade acetonitrile and flow rate was set on 1 ml/minute at 271 nm wave length. The retention time for etravirine was found to be 1.80 minutes.

Result: The method was found to be linear in the range of 10-60 µg/ml. In the linearity study, regression equation and correlation coefficient were found to be y=16.95x+17.148 and 0.999, respectively. This method was rugged and robust in different testing criteria, limit of detection and limit of quantification was found to be 0.514 µg/ml and 1.713 µg/ml respectively. Accuracy study was carried out in three different concentration level i.e. 50, 100, 150% and % recovery of the method was found to be 98.6%, 99.08%, 99.17% respectively in three different levels and mean recovery was 98.95% and hence method was accurate.

Conclusion: Results of all validation parameter were within the limits as per International Conference on Harmonization guideline.

Keywords: High performance liquid chromatography, Validation, Method development, Artemether, Accuracy, Precision.

INTRODUCTION

Etravirine is a substituted diaryl pyrimidine derivative human immunodeficiency virus (HIV). It is a secondary non-nucleoside reverse transcriptase inhibitor that directly bine active against ds to reverse transcriptase enzyme and blocks the catalytic actions of the enzyme, which regulates the replication of the genetic material of HIV [1]. Etravirine is active against wild type HIV-1 and approved by Food and Drug Administration as anti-AIDS agent. Chemically it is 4-[[6-amino-5-bromo- 2[(4-cyanophenyl)-amino] pyrimidinyl] oxy]-3, 5-dimethylbenzonitrile [2]. Literature survey [3-8] reveals that there are very few analytical methods for analysis of etravirine in different formulations out of these methods only three method are in high performance liquid chromatography (HPLC). Hence, the present study was aimed to develop a simple and accurate reverse phase (RP) HPLC method for the routine analysis of etravirine (Fig. 1).

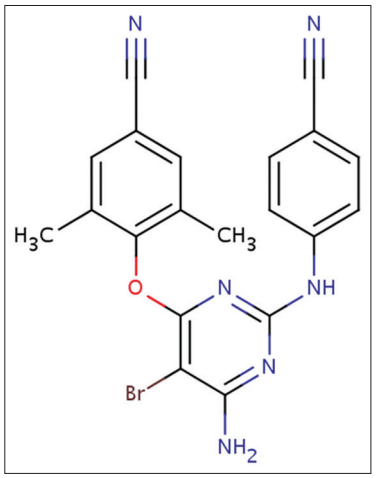

Fig. 1: Structure of etravirine

METHODS

Standard drugs

Etravirine was procured from the HETERO Pharma.

Chemicals and reagent

Methanol (Finer Chemical Ltd.), acetonitrile (Rankem Chemicals), purified water (Rankem Chemicals).

Instruments

HPLC (Analytical Technologies), ultraviolet (UV) (Elico SL-196), detector (UV detector, Analytical Technologies), column (hypersil ods C18, (150*4.6 mm, 5 µ), software (Analchrome, Clarity), sonicator (Analytical Technologies).

Preparation of mobile phase

Accurately measured 500 ml (100%) of acetonitrile HPLC grade was degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ nylon filter under vacuum filtration.

Diluent

Mobile phase is used as diluents.

Standard preparation

Accurately weigh 25 mg of etravirine and transfer in to 25 ml volumetric flask. Add about 10 ml of solvent sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent. Transfer 1 ml of above solution in to a 10 ml volumetric flask and dilute to volume with diluent, and again transfer 4 ml of above solution in to a 10 ml volumetric flask and dilute to volume with diluent. Standard chromatogram is given in Fig. 2.

Sample preparation
Accurately weigh 25 mg of etravirine and transfer in to 25 ml volumetric flask. Add about 10 ml of solvent sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent. Transfer 1 ml of above solution in to a 10 ml volumetric flask and dilute to volume with diluent, and again transfer 4 ml of above solution in to a 10 ml volumetric flask and dilute to volume with diluent. Sample chromatogram is given in Fig. 3.

Optimized chromatographic conditions (Fig. 4)

Column	Hypersil ods C18 (150*4.6 mm), 5 µ
Flow rate	1 ml/minute
Wavelength	271 nm
Column temperature	35°C
Injection volume	10 µl
Run time	5 minutes

Method validation
The following parameters were considered for the analytical method validation of etravirine in bulk form.

System suitability
Chromatograph of the standard preparations (six replicate injections) and peak area responses for the analyte peak was measured, and the system suitability parameters are evaluated.

Accuracy
For accuracy determination, three different concentrations were prepared separately, i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Precision
The standard solution was injected for 6 times and the area was measured for all six injections in HPLC. The % relative standard deviation (RSD) for the area of six replicate injections was found to be within the specified limits.

Robustness
As part of the robustness, deliberate change in the temperature and flow rate variation was made to evaluate the impact on the method.

Linearity and range
Linearity of the analytical method for assay by injecting the linearity solutions prepared in the range of 10-60 µg (25-150%) of test concentration, into the chromatograph, covering minimum six different concentrations.

Ruggedness
Establish the ruggedness of the analytical method by using the assay of six different sample preparations of the same batch by a different analyst using a different HPLC system.

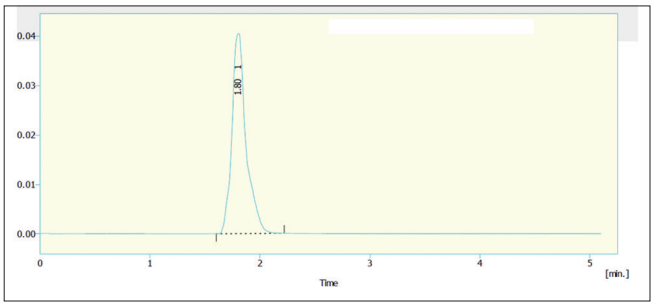

Fig. 2: Standard chromatogram of etravirine

RESULTS AND DISCUSSION

Standard preparation
Accurately weigh 25 mg of etravirine and transfer in to 25 ml volumetric flask. Add about 10 ml of the solvent mixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with a solvent mixture. Transfer 1 ml of above solution in to a 10 ml volumetric flask and dilute to volume with diluent, and again transfer 4 ml of above solution in to a 10 ml volumetric flask and dilute to volume with diluent.

Validation

Accuracy
Average recoveries of etravirine are 101.6%, 99.6%, 99.7%, at 50%, 100% and 150% concentrations level respectively. The percentage recoveries of the drug are within the limits 99-102%. Hence, the method is accurate, accuracy data for etravirine are presented in Table 1. % recovery was found to be 98.85% as mentioned in Table 2.

Precision
Precision are summarized in Table 3 respectively. The % RSD values for the precession was <2.0%, which indicates that the proposed method is precise.

Linearity
The response was found linear over a concentration range of 10-60 µg/mL of etravirine. The correlation co-efficient were found to be 0.999 for etravirine. Hence, the method is linear, data is presented in Table 4. Linearity curve of etravirine is given in Fig. 5.

Robustness
Minor deliberate changes in different experimental parameters such as flow rate (±0.2 ml) and temperature (±5°C) did not significantly

Table 1: Accuracy results of etravirine

Concentration level	Amount added (mg)	Amount found (mg)	% Recovery	Average % recovery
50%	12.5	12.3	98.4	98.6
		12.3	98.4	
		12.4	99.2	
100%	25	24.95	99.02	99.08
		24.97	99.1	
		24.98	99.13	
150%	37.5	37.3	99.4	99.17
		37.4	99.22	
		37.2	98.90	

Table 2: % Recovery of etravirine

Amount added (mg)	Amount found (mg)	Average % recovery
25	24.95	98.85

Table 3: Precision results of etravirine

Sample. no	Peak area of etravirine
Injection 1	532.054
Injection 2	534.140
Injection 3	529.788
Injection 4	526.325
Injection 5	533.284
Injection 6	533.232
Mean	531.4705
SD	2.9382080436
%RSD	0.55

RSD: Relative standard deviation

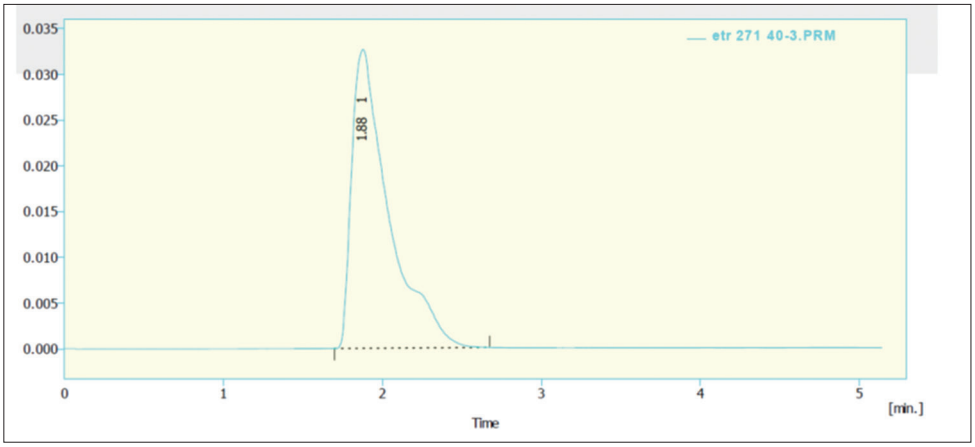

Fig. 3: Sample chromatogram of etravirine

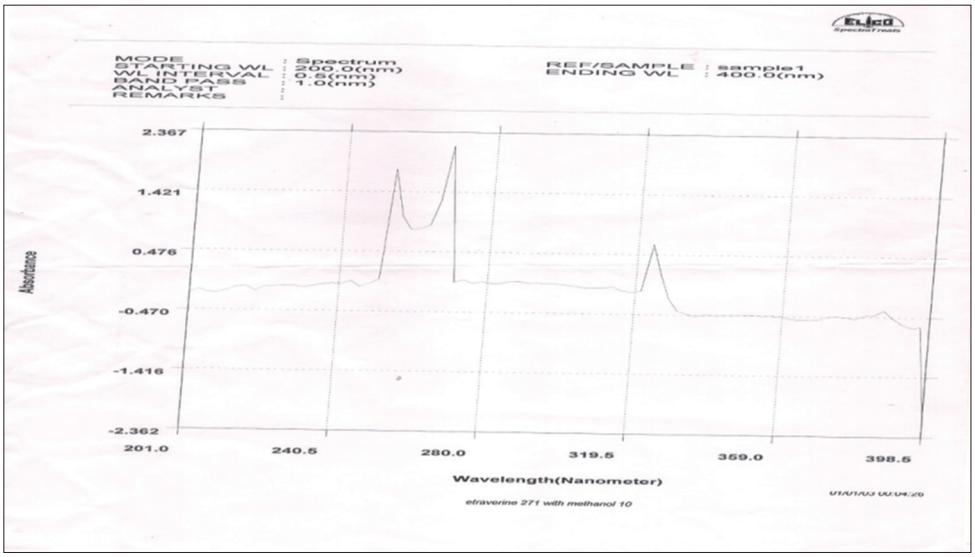

Fig. 4: Ultraviolet spectrum of etravirine

Table 4: Linearity results of etravirine

% Level	Concentration (µg/ml)	Peak area
25	10	164.307
50	20	342.28
75	30	500.38
100	40	669.588
125	50	844.534
150	60	1029.54
Y intercept		16.95
Correlation co-efficient (r^2)		0.999
Slope		17.1489
Linearity range	10-60	

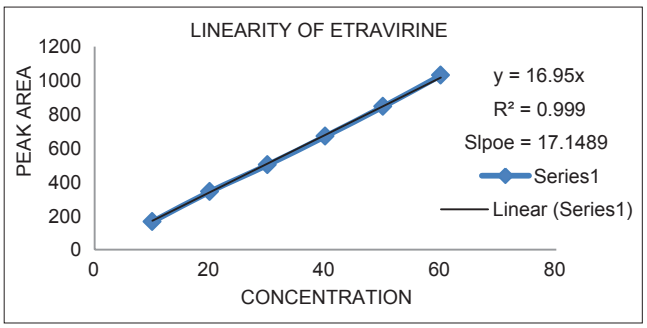

Fig. 5: Calibration graph of etravirine

affect the retention time and peak area of etravirine indicating that the proposed method is robust which is mentioned in Tables 5 and 6.

Ruggedness

The method is rugged by different time intervals and the method did not significantly affect the recoveries, peak area and retention time of all the above drugs indicating that the proposed method is rugged, which is mentioned in Table 7.

Limit of detection (LOD)

The LOD is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected, The LOD are calculated by formula:

LOD=3.3×SD/b

Where, SD - standard deviation of the peak area of the drugs, b - is the slope of the corresponding calibration curve. LOD for etravirine was 0.514 µg/ml.

Limit of quantification (LOQ)

The LOQ is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at

Table 5: Robustness results of etravirine (change in flow rate)

S. no	Flow rate	Peak area of etravirine		Average	SD	% RSD
1	0.8 ml/minutes	282.208	285.301	283.75	2.1870	0.77
2	1 ml/minutes	362.597	353.80	358.198	6.220	1.73
3	1.2 ml/minutes	324.453	331.65	328.051	5.089	1.55

RSD: Relative standard deviation

Table 6: Robustness results of etravirine (change in temperature)

S. no	Temperature	Peak area of etravirine		Average	SD	% RSD
1	30°C	470.584	476.412	473.498	4.1210	0.87
2	35°C	362.597	353.80	358.198	6.220	1.73
3	40°C	529.788	524.673	527.230	3.616	0.685

RSD: Relative standard deviation

Table 7: Ruggedness results of etravirine

Name	Peak area of etravirine
Ruggedness (Day-1)	410.383
Ruggedness (Day-2)	404.763
Ruggedness (Day-3)	407.399
Ruggedness (Day-4)	402.921
Ruggedness (Day-5)	409.509
Ruggedness (Day-6)	406.637
Average	406.9353
SD	2.81595
% RSD	0.691

RSD: Relative standard deviation

which the analyte can be quantified with acceptable accuracy and precision, The LOQ are calculated by formula:

LOQ=10×SD/b

Where, SD - standard deviation of the peak area of the drugs, b - is slope of the corresponding calibration curve. LOQ for etravirine was 1.713 µg/ml (Table 8).

CONCLUSION

Method development and validation of etravirine was done by RP-HPLC method. The estimation was done by using hypersil C_{18} (4.6 mm×150 mm, 5 µm, make: Analytical Technologies). Mobile phase was used as acetonitrile at a flow rate 1 ml/minute, retaintion time was 1.8 minutes. At λ max 271 nm. The linearity range of etravirine was found to be 10-60 µg/ml. Mean recovery was 98.85%, which is within 98-102%. Correlation coefficient value was 0.999, % RSD was 0.55%, which is within the limit. Validation summary is given in Table 9. These results show the method is accurate, precise, sensitive, economic and rugged. The HPLC method is rapider. The proposed method can be successfully applied to estimate bulk drug and tablet dosage form. The method was found to be having suitable application in routine laboratory analysis with a high degree of accuracy and precision.

Table 8: LOD and LOQ results of etravirine

Parameters	Artemether
LOD	0.514 µg/ml
LOQ	1.713 µg/ml

LOD: Limit of detection, LOQ: Limit of quantification

Table 9: Validation summary of etravirine

S. no	Parameter	Acceptance criteria	Obtained results
1	Theoretical plates	NLT 200	8100
2	Accuracy	Recovery 98-102%	98.85%
3	Precision	% RSD NMT2	0.55%
4	Specificity	No interference	No impurity
5	Linearity		10-60 µg/ml
6	Ruggedness	% RSD NMT 2	0.691
7	LOD		0.514 µg/ml
8	LOQ		1.713

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

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